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The no-reflow phenomenon: A basic mechanism of myocardial ischemia and reperfusion

Abstract Both animal models of experimental myocardial infarction and clinical studies on reperfusion therapy for acute myocardial infarction have provided evidence of impaired tissue perfusion at the microvascular level after initiation of reperfusion despite adequate restoration of epicardial vessel patency. Characteristics of this "no-reflow" phenomenon found in basic science investigations, such as distinct perfusion defects, progressive decrease of resting myocardial flow with ongoing reperfusion and functional vascular alterations are paralleled by clinical observations demonstrating similar features during the course of reperfusion. In experimental animal investigations of coronary occlusion and reperfusion, this no-reflow phenomenon could be characterized as a fundamental mechanism of myocardial ischemia and reperfusion. Major determinants of the amount of no-reflow are the duration of occlusion, infarct size, but also the length of reperfusion, as rapid expansion of perfusion defects occurs during reperfusion. Moreover, no-reflow appears to persist over a period of at least four weeks, a period when major steps of infarct healing take place. The significant association of the degree of compromised tissue perfusion at four weeks and indices of infarct expansion, found in chronic animal models of reperfused myocardial infarction, might be the pathoanatomic correlate for the prognostic significance observed in the clinical setting.

Key words no-reflow – ischemia – reperfusion – myocardial blood flow – myocardial infarction

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Introduction

Timely reperfusion therapy is the current treatment option of choice for ST-segment elevation myocardial infarction [72, 84], as an occlusive thrombus in the epicardial coronary artery appears to be the underlying cause in the vast majority of patients with acute myocardial infarction [16]. Currently, the debate on myocardial reperfusion in the clinical realm primarily focuses on whether primary percutaneous coronary angioplasty (PTCA) may be more advantageous than pharmacologic thrombolysis and how to improve patients' access to this treatment within an adequate time window [48].

However, *reperfusion* therapy, i. e. restoration of blood flow to the previously ischemic myocardium, may be a much more challenging treatment concept than suggested by the widespread and obviously successful application of PTCA and thrombolysis, usually called reperfusion therapy. As early as the 1960s, studies performed in experimental animal models demonstrated that ligation of a coronary artery and subsequent reopening of the epicardial vessel may result in incomplete reperfusion of the microvascular bed despite adequate restoration of epicardial vessel diameters [59]. Distinct zones of hypoperfusion, characterized by severely depressed myocardial blood flow, may develop within the previously ischemic myocardial tissue. Based on these observations, the term "no-reflow" was coined, describ-

ing compromised myocardial blood flow at the microvascular level after coronary artery occlusion and reperfusion [53].

Moreover, in recent years, a growing number of clinical investigations, using imaging techniques to visualize myocardial perfusion after reperfusion therapy for acute myocardial infarction, demonstrated distinct zones of markedly compromised perfusion within the myocardium supplied by the coronary artery which had been re-opened [38, 100, 106, 107]. These clinical observations of reduced tissue perfusion in spite of restoration of epicardial coronary flow shared many similarities with the no-reflow phenomenon, first delineated in experimental animal models. Therefore, the term no-reflow was also used for clinical perfusion defects after reperfusion therapy for acute myocardial infarction [41], notwithstanding that some clinical aspects of no-reflow may also substantially differ from experimental models of coronary occlusion and reperfusion [15, 98]. Coronary microembolization which significantly contributes to microvascular perfusion deficits in the clinical context is not simulated in animal models of coronary occlusion and reperfusion [20, 86]. Interestingly, the amount of no-reflow, as visualized by myocardial contrast echocardiography, scintigraphic techniques or magnetic resonance imaging in the clinical realm, has reproducibly been shown to significantly predict left ventricular segmental wall motion dysfunction, ventricular remodeling and clinical outcome [68, 76, 96, 107], which initiated a body of clinical research on prognostic aspects and potential therapeutic options for clinical no-reflow.

Therefore, pathophysiologic aspects of no-reflow, whether seen from the clinical point of view as a promising target of treatment to fulfill the high demands of true reperfusion therapy or from the light of basic science as a fundamental mechanism of myocardial ischemia and reperfusion, may become a novel or revived focus of research.

This review summarizes the main characteristics, pathophysiologic models, potential interventions and consequences of the no-reflow phenomenon, as seen in experimental models of coronary occlusion and reperfusion. Also, differences and similarities of the clinical phenomenon are discussed in order to provide the basic science counterpart within the synopsis on different aspects of the no-reflow phenomenon compiled in this series of *Basic Research in Cardiology*.

Major characteristics of the no-reflow phenomenon

After re-opening of an occluded artery, reperfusion of the previously ischemic tissue depends on microvascular integrity. The "no-reflow" phenomenon may be defined as incomplete and non-uniform reperfusion at the microvascular level despite adequate re-opening of the proximal artery after a period of transient ischemia. First described after cerebral ischemia in 1967 [63], the no-reflow phenomenon was demonstrated in several other organs after temporary arterial occlusion, such as the skin [105], the kidney [95] and also in the heart [53, 59].

In animal models of coronary occlusion and reperfusion, the presence of distinct areas of impaired perfusion within the previously ischemic myocardium, marked ultrastructural damage of the microvasculature related to coronary ischemia and reperfusion, and a progressive reduction of regional myocardial blood flow [5, 53] were identified as the major characteristics of the myocardial no-reflow phenomenon.

Anatomical area of no-reflow

In order to visualize areas of impaired perfusion after coronary artery occlusion and reperfusion, different dyes were injected into the coronary circulation (without re-occlusion of the coronary artery) in several animal studies of experimental myocardial infarction. The area not stained by the dye was defined as the area of no-reflow [5, 53] (Fig. 1). In a canine heart, which is characterized by relatively high collateral flow to the ischemic tissue during occlusion, intra-atrial injection of the dye carbon black (20–30 nm particles) and intravenous injection of thioflavin S, a fluorescent vital stain for the endothelium, after 40 minutes of coronary occlusion and reperfusion revealed a homogenous distribution of both dyes [53]. However, after 90 minutes of occlusion of the circumflex coronary artery with subsequent reperfusion, areas of no-reflow were identified as tissue not stained by carbon black and thioflavin S. These areas of no-reflow were predominantly located in the subendocardial myocardium. Non-fluorescent areas and carbon black-negative areas revealed marked ultrastructural damage of the microvasculature upon electron microscopy. As expected, the area of no-reflow determined by injection of thioflavin S (non-fluorescent area) was characterized by low regional myocardial blood flow at the time of perfusion-staining, ranging from 0.13 to 0.39 ml/g/min, as assessed by radioactive microspheres [5, 44]. In general, non-ischemic regional myocardial blood flow amounts to approximately 1.0 ml/g/min in the canine heart.

Also in a rabbit heart, which in contrast to the dog has negligible collateral flow to myocardial tissue after coronary occlusion, simultaneous intra-atrial injection of Uniperse blue, a particulate blue dye (similar to Monastral blue), and thioflavin S demonstrated closely correlating areas not stained by the two dyes after various durations of occlusion and reperfusion. However in these experiments, the thioflavin S-negative area was always

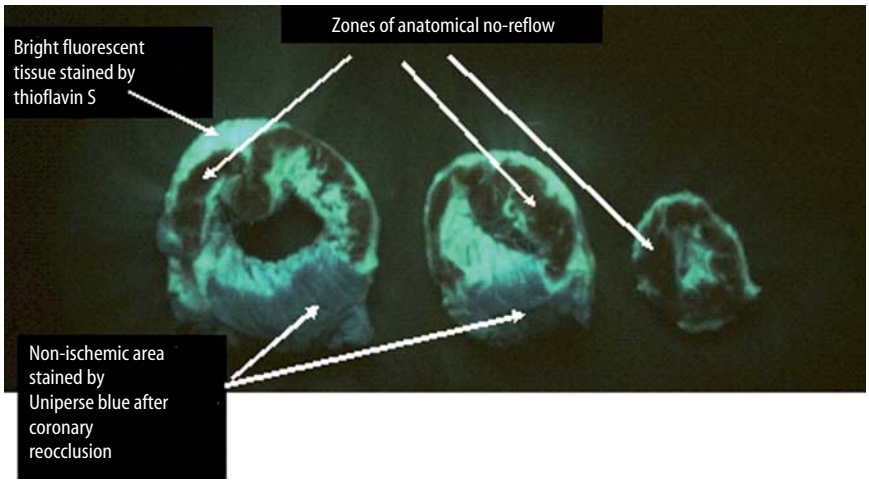

Fig. 1 Three apical slices of a rabbit heart after 30 min of coronary occlusion and 120 min of reperfusion. At the end of reperfusion Thioflavin S, a vital fluorescent stain for endothelium was injected without coronary re-occlusion in order to stain perfused tissue. Thereafter, the coronary artery was re-occluded, and 50% Uniperse blue, a particulate blue dye, was injected to delineate the ischemic risk area. Under fluorescent light the reperfused myocardium appears bright, whereas the anatomical no-reflow zone remains dark. The tissue stained by the blue dye is the non-ischemic myocardium, whereas the tissue not stained by the blue dye represents the area at risk

slightly smaller and confined to the area not stained by Uniperse blue regardless of the duration of occlusion and reperfusion [83]. Thus, this so-called anatomical no-reflow represents zones of severe regional myocardial hypoperfusion related to microvascular injury. The exact size of the visualized areas of no-reflow depends on the specific ability of the injected dye to penetrate into the tissue with incomplete reflow.

Recently, in vivo assessment of myocardial perfusion defects also became feasible with myocardial contrast echocardiography, magnetic resonance imaging and scintigraphic techniques. In a canine model of coronary occlusion and reperfusion, contrast-echocardiography demonstrated myocardial perfusion defects as regions of lower myocardial contrast enhancement after injection of echocardiographic contrast medium. These myocardial areas were associated with low myocardial blood flow as determined by radioactive microspheres and closely correlated with infarct size [100]. In contrast-enhanced myocardial magnetic resonance imaging (MRI), zones of hypoenhancement (after application of gadolinium-contrast medium) after myocardial infarction are considered to represent areas of microvascular obstruction and correspond well to anatomical no-reflow zones determined by injection of thioflavin S and no-reflow as visualized by contrast echocardiography [44, 106]. Similarly, anatomical no-reflow and its close relation to thioflavin S-negative zones was demonstrated by ^{82}rubidium positron emission tomography [42].

Moreover, other experimental approaches provided evidence for distinct compartments of reflow and of no-reflow after coronary artery occlusion and reperfusion, as well. For example, by means of ^{31}P-nuclear magnetic resonance spectroscopy, different compartments of the myocardial tissue according to differing pH-levels could be separated after various times of ischemia and reperfusion, indicating that these different compartments, i. e. the no-reflow area and the tissue with reflow, are functionally separated [34].

Ultrastructural alterations of the microvasculature

Zones of no-reflow, as assessed anatomically on a macroscopic basis, are characterized by specific ultrastructural changes, when examined by electron microscopy. In a dog model of coronary artery occlusion, marked ultrastructural capillary damage after 90 minutes of coronary artery occlusion with and without initiation of reperfusion was demonstrated [53]: Swollen intraluminal endothelial protrusions and membrane-bound, in part free-floating bodies within the lumen (blebs) were common. These areas of regional swelling often seemed to obstruct the capillary lumen. The endothelial cells showed decreased numbers of pinocytotic vesicles and chromatin margination.

When the coronary artery had been re-opened after a certain duration of occlusion, many capillaries in areas not perfused by thioflavin S contained tightly packed red blood cells. Endothelial protrusions and membrane-bound bodies often filled the capillaries to a point that the lumen was obliterated. After 20 minutes of reperfusion, large endothelial gaps with extravascular erythrocytes were demonstrated in these areas. Occasionally, capillaries appeared to be compressed by subsarcolemmal blebs. These changes were not observed after 40 minutes of occlusion (when no anatomical no-reflow areas had developed) or in thioflavin S-positive areas and tissue samples not supplied by the previously occluded artery. Fishbein et al. demonstrated a close correlation of myocardial hemorrhage to vascular injury [21]. The hemorrhage was always confined to the area of vascular damage (not stained by carbon black in these investigations). In addition, these areas were predominantly located in the subendocardium and within the area of

necrosis. After different durations of coronary occlusion (20–180 minutes) in dogs, ultrastructural evidence of microvascular damage with intraluminal blebs (beginning after 60 minutes occlusion), as delineated above, always lagged behind myocardial cell injury, which was already visible after 20 minutes of ischemia [55].

Myocardial blood flow during reperfusion and functional vascular alterations

As can be expected, morphological findings were accompanied by a reduction of regional myocardial blood flow within the previously ischemic area. As a standard method, experimental models most often used injection of radioactive or colored microspheres with simultaneous withdrawal of a reference blood sample to assess myocardial blood flow. Anatomical areas of no-reflow in different studies in the dog always revealed low regional blood flow between 0.13 and 0.39 ml/g/min [5, 44], which is substantially depressed in relationship to regional flow in the non-ischemic zone (about 1 ml/g/min). In a study by Ambrosio et al., blood flow within the risk area (area supplied by the coronary artery that had been re-opened) tended to be hyperemic in the first minutes after release of a coronary occlusion followed by a progressive decrease in flow during 3.5 hours of reperfusion that was more pronounced in the subendocardium [5]. This progressive decrease in myocardial tissue perfusion was not related to variation in perfusion pressure, but due to progressive microvascular damage.

Similarly, in the rabbit, 30 minutes of coronary occlusion followed by reperfusion resulted in hyperemic blood flow in the ischemic risk area at 2 minutes of reperfusion (3.78 ml/min/g), which then progressively deteriorated, reaching a plateau of approximately 0.9 ml/min/g at 2 hours of reperfusion [79]. Normal regional blood flow in the rabbit amounts to 2.06 ml/min/g.

Albeit not included into the definition of the no-reflow phenomenon, functional vascular alteration in response to ischemia/reperfusion may be important when transferring the complex of no-reflow and microvascular damage into the clinical context. Therefore, it should be mentioned that, in addition to compromised resting myocardial blood flow, the no-reflow phenomenon is accompanied by an altered response of the vasculature to various dilatory stimuli probably involving reactive oxygen species and leukocyte-mediated mechanisms: To mention only an example among the extensive work in this field, endothelium-dependent relaxation, characterized by a nitric oxide-dependent pathway in response to acetylcholine, was markedly reduced in isolated rat coronary arteries subjected to ischemia and reperfusion, but not after ischemia alone [45, 54, 82], indicating also functional damage induced by ischemia and reperfusion.

Major determinants of anatomic no-reflow

Duration of coronary occlusion

Even in the early investigations in the dog, some fundamental determinants of the development of no-reflow were demonstrated: A certain threshold of the duration of ischemia seemed to be necessary for the development of zones of no-reflow. In the canine experiments (characterized by relatively high collateral flow) described above, 40 minutes of ischemia did not lead to perfusion defects after 20 minutes of reperfusion, but substantial areas of no-reflow were visualized after 90 minutes of ischemia with reperfusion for 10 seconds to 20 minutes [53]. Thereafter, the extent of microvascular damage worsened with longer durations of ischemia [55].

Size of myocardial necrosis

In the rabbit heart (characterized by negligible collateral flow), a similar relationship was demonstrated with bigger areas of no-reflow after longer durations of coronary occlusion. A closer look at these investigations, however, demonstrated that the amount of necrosis, as determined by triphenyltetrazolium chloride, and the degree of reflow to the previously ischemic zone were closely correlated with each other after various durations of coronary occlusion and reperfusion. Smaller infarcts were associated with higher regional myocardial blood flow within the area at risk and vice versa at any given time point of reperfusion [78]. Comparably, the size of myocardial infarction was closely correlated with the amount of anatomical no-reflow visualized by perfusion staining with thioflavin S along with a high spatial concordance [79, 80].

While, in these experimental series, the no-reflow zone was confined to the zone of infarction and tended to be slightly smaller in size in the majority of hearts, infarct size seemed to be the major determinant of the amount of no-reflow irrespective of the duration of coronary occlusion. In following experimental series, this correlation was a very consistent and reproducible result [29, 31, 78–82]. Thus, the observation that longer durations of occlusion tend to result in bigger no-reflow zones may potentially solely be explained by bigger infarcts after longer durations of ischemia. Thus, infarct size is a major determinant of the size of anatomical no-reflow.

In these experiments, ischemic preconditioning, one of the most powerful mechanisms of infarct size reduction in animal research, reduced myocardial necrosis

and microvascular perfusion defects in a proportional fashion [80], which further strengthens the concept of necrosis as a major determinant of microvascular damage. Interestingly, also postconditioning, i. e. the application of short periods of re-occlusion after ischemia and reopening of the epicardial arteries, also appears to reduce infarct size [93, 108, 109]. Experimental data also suggest that vascular functioning can be improved by postconditioning, along with reduced leukocyte accumulation and greater maximal vasodilatory responses [108]. Investigations in patients using short periods of recurrent balloon inflation after percutaneous transluminal coronary angioplasty to mimic postconditioning also suggested reduced infarct size and also increased blush grade, a parameter used in clinical circumstances to describe microvascular perfusion [93].

Duration of reperfusion

Ambrosio et al. demonstrated a more than twofold increase of the size of no-reflow zones from 2 minutes of reperfusion to 3.5 hours of reperfusion in the canine. In these experiments, the area not stained by thioflavin S expanded from the subendocardium towards the epicardial myocardium. This progression of anatomical no-reflow during the time course of reperfusion, which was accompanied by a progressive reduction of regional myocardial blood flow [5], can be interpreted as reperfusion injury at the microvascular level [42, 88].

In order to define the time course of reperfusion-related progressive microvascular damage, anatomic no-reflow and regional myocardial flow were determined after 30 minutes of coronary occlusion and 2, 30, 60 and 480 minutes of reperfusion in the rabbit [79]. At two minutes of reperfusion, the area of no-reflow amounted to 12.2 % of the risk area; thereafter a progressive increase in the no-reflow zone to 30.8 % after 2 hours of reperfusion and 34.9 % after 8 hours was observed. Along with a reduction of regional myocardial flow in the risk area from hyperemic values after two minutes of reperfusion to approximately 0.9 ml/g/min at 2 hours and 8 hours of reperfusion, the anatomical zone of no-reflow appeared to reach a plateau after two hours without further significant deterioration by 8 hours. Size and spatial distribution of no-reflow and macroscopically visible hemorrhage closely correlated with each other in these experiments. With regard to longer observational periods after the insult of ischemia and reperfusion, data are in part contradictory. While it seems to be established that major characteristics of no-reflow persist within the infarcted myocardial tissue for at least 4 weeks in the rat [77], the exact time-course within the first hours and days is different in various studies, presumably in part due to species differences. Using scintigraphic techniques (^{82}Ru positron emission tomography) a progressive deterioration of microvascular flow was demonstrated in the first four hours of reperfusion in the dog [42]. In the canine model of myocardial infarction used in the MRI investigations by Rochitte et al. the zone of microvascular obstruction increased from 13 % at 2 hours of reperfusion to 23 % at 6 hours of reperfusion with a further increase to 30 % at 48 hours of reperfusion [88]. However, even within the same species, there are significant differences in the amount of no-reflow at various time points of reperfusion when analyzed at a quantitative level [44]. It should be added that also in the clinical arena, convincing evidence for reperfusion-associated progression of microvascular perfusion defects exists, which in part may be related to similar mechanisms [41, 58]. Interestingly, also in the clinical realm, evidence for persistence of perfusion defects was provided by contrast echocardiography over the first month after reperfused myocardial infarction [6]. In summary, the duration of reperfusion is a significant determinant of the amount of no-reflow, probably representing a form of reperfusion injury at the microvascular level. Systematic evaluation regarding the time-course of this reperfusion injury suggested that the most significant amount of microvascular deterioration occurs during the first hours after initiation of reperfusion, presenting a potential time-window for therapeutic interventions.

Potential mechanisms of no-reflow

While the exact mechanisms responsible for anatomical no-reflow, as visualized in animal studies of coronary occlusion and reperfusion, have not been completely understood some potential causal factors as well as hypotheses that have been put forward will be discussed below.

Endothelial ischemic damage and microvascular obstruction

The pronounced ultrastructural abnormalities of the capillary endothelium demonstrated by electron microscopy suggest that morphological features of microvascular damage related to ischemia may directly contribute to the no-reflow phenomenon. Localized areas of endothelial swelling and numerous endothelial protrusions were the most common findings in the already mentioned initial ultrastructural investigations in the dog [53]. These protrusions, which resemble blisters forming upon the endothelial surface, may act to occlude the capillary lumen and thus play a direct role in causing regional perfusion defects. Following reperfusion, capillaries showed tightly packed erythrocytes, suggesting that some flow must have occurred initially

into these regions after release of the coronary occlusion. Endothelial gaps were present after 20 minutes of reflow and, occasionally, fibrin and platelet thrombi were demonstrated in the microvasculature. The experiments by Ambrosio et al. revealed similar morphologic changes of the vascular endothelium with signs of red blood cell stasis in the thioflavin S negative areas both after 2 minutes of reperfusion and after 3.5 hours of reperfusion. In addition, after 3.5 hours, there was a striking accumulation of neutrophils within the microvasculature that appeared to obstruct the vascular lumen in areas not stained by thioflavin S [5].

Leukocyte plugging

Engler et al. further explored the neutrophil plugging concept as a mechanistic explanation of no-reflow. After coronary occlusion and reperfusion, they observed extensive leukocyte plugging that could not be washed out by crystalloid perfusion in capillaries within areas of no-reflow in comparison to capillaries from areas with reflow [19]. This might be a cause of erythrocyte packing and rouleaux formation upstream from the mechanical obstruction with subsequent changes in local hematocrit, erythrocyte flexibility and blood viscosity. Perfusion with leukocyte-depleted solutions [22, 61, 85] or reperfusion with oxygenated perfluorochemicals [30, 57] led to a reduction of the anatomical area of no-reflow in various studies. In this context, it should be emphasized that even in isolated saline-perfused Langendorff hearts an anatomical area of no-reflow can be visualized [60]. Thus leukocytes may enhance myocardial no-reflow, but they are not essential.

Mechanical compression

Sudden myocardial cell swelling with prominent intracellular and interstitial edema is one of the very early morphologic changes induced by reperfusion and is probably in part linked to osmotic forces due to glycolytic pathway products [99]. As tissue edema might compress the microvascular bed, the no-reflow phenomenon may in part be attributed to changes in total cross sectional vascular area [64]. However, increasing serum osmolality by administration of mannitol did not exhibit beneficial effects on the amount of no-reflow in all experimental designs [13, 101]. Moreover, progression of the area of no-reflow with ongoing reperfusion is not likely to be due to microvascular compression alone, as tissue edema largely develops immediately after reperfusion.

Interaction between leukocytes, platelets, and the endothelium

Even if capillary leukocyte trapping is prominent in the area of no-reflow, the effects of leukocytes probably are not solely confined to mechanical plugging, but may involve complex interactions with the endothelium, platelets and perhaps with myocytes. An influx of leukocytes into the arterial wall, accompanied by reduced endothelium dependent and independent vasodilation, was shown after three hours of coronary occlusion with subsequent reperfusion [54]. Polymorphonuclear leukocytes are able to release reactive oxygen metabolites, such as hydrogen peroxide [69] and hypochloric acid as a product of the myeloperoxidase reaction [103], and proteolytic enzymes, such as elastase and lipooxygenase products (leukotrienes) that exhibit influence on platelet and endothelial function [90]. For example, oxygen radicals, released by neutrophils, could damage the endothelial surface with subsequent regional swelling and blebbing. Endothelial cells can modulate leukocyte function by the expression of adhesion molecules and by release of soluble factors including nitric oxide, prostacyclin, endothelins, platelet activating factor and interleukin-8. In cultured endothelial cells, increased expression of the intercellular adhesion molecule-1 (ICAM-1) was observed after 6 hours of anoxia, which was prevented by a preconditioning stimulus or a free radical scavenger, and was accompanied by increased adhesion of leukocytes to the endothelial cells [9]. The contribution of P-selectin, which was shown to be expressed on postischemic endothelium and to trigger leukocyte attachment, to the no-reflow phenomenon remains controversial. In isolated heart preparations, perfused with leukocyte-supplemented buffer, a beneficial effect of a P-selectin antagonist was demonstrated [71], whereas in an open-chest model (in vivo model with full-blood perfusion) no effect on regional myocardial flow after reperfusion was apparent [10]. Platelets affect polymorphonuclear cell activation by release of thromboxane A2, platelet derived growth factor, serotonin, lipooxygenase products, proteases and adenosine. In hypercholesterolemic rabbits, a marked platelet accumulation has been described with enhancement of no-reflow that could be avoided by platelet depletion [25]. Thus, in addition to their direct involvement in the development of no-reflow, neutrophils, platelets and endothelial cells are linked in a complex functional balance that is disturbed by ischemia and reperfusion.

The complement cascade may further modulate these interactions. Activation of neutrophils by the activated factor C5a of the complement system was shown to produce myocardial ischemia when given directly into the coronary artery [36, 65]. The production and the release of endothelin during myocardial ischemia and reperfusion are markedly enhanced, which may con-

tribute to increased vascular resistance. Endothelin receptor antagonism was shown to reduce anatomical no-reflow as well as functional vascular injury [23, 61]. In conclusion, there may be multiple interactions of leukocytes, endothelium, platelets, and soluble as well as cell-related mediators that contribute to the mechanism of no-reflow.

Reactive oxygen species

The production of oxygen free radicals peaks during the first 2 to 10 minutes of reperfusion after coronary artery occlusion. Besides the effect of polymorphonuclear cells as a source of reactive oxygen species, the endothelial xanthine oxidase and potentially cardiac myocytes may be a source of superoxide anions, derived from the oxidation of accumulated hypoxanthine [14]. Recent investigations suggest that the major amount of free oxygen radicals is generated by neutrophils during reperfusion [18]. While superoxide anions are normally catalyzed to hydrogen peroxide by superoxide dismutase, this pathway is markedly altered after an ischemic insult, thus, contributing to the burst of reactive oxygen specimen upon reperfusion [28].

Several investigations have shown that administration of superoxide dismutase in combination with catalase reduces the degree of no reflow, the area of no-reflow and ultrastructural signs of endothelial injury [1, 3, 75, 101]. Przyklenk et al. demonstrated in a canine model of 2 hours of coronary occlusion followed by 4 hours of reperfusion that administration of superoxide dismutase and catalase during reperfusion significantly improved regional myocardial blood flow during reperfusion and preserved microvascular ultrastructure. Interestingly, in this study, oxygen radical scavenging did not significantly reduce infarct size, but preserved microvascular ultrastructure in zones of severely damaged cardiomyocytes. There is still controversy regarding whether myocardial infarct size can be reduced when the radical scavengers are given only during reperfusion [3, 4, 75, 101]. Nevertheless, these studies provide strong evidence that microvascular damage during reperfusion is caused at least in part by reactive oxygen species.

Coagulation, tissue factor

Ultrastructural investigations of no-reflow areas did not show direct evidence of a causal role of intravascular thrombus formation for the development of no-reflow in animal models of mechanical coronary artery occlusion and reperfusion. Some electron microscopy studies found rare micro thrombi [53]; others did not find any evidence for activation of the coagulation pathway [59]. Administration of acetylsalicylic acid [43], streptokinase [51] or tissue-plasminogen activator [52] did not demonstrate any beneficial effects on microvascular integrity. But in hypercholesterolemic rabbits, platelet accumulation seemed to contribute to the extent of no-reflow [25]. Some experimental observations suggest that tissue factor, a membrane-bound glycoprotein that activates the extrinsic coagulation pathway (via activating factor VII) when exposed to flowing blood, contributes to the degree of no-reflow. When active site-blocked factor VII was administered during reperfusion, a marked reduction of no-reflow, along with infarct size reduction, was observed, whereas activated factor VII led to deterioration of the no-reflow phenomenon [26, 27]. In addition, this study demonstrated intracoronary accumulation of fibrin/fibrinogen and platelets in the ischemic area, which was attenuated by active site-blocked factor VII. Oxygen free radicals stimulate the synthesis of tissue factor, thereby providing a potential concept for its involvement in no-reflow [26]. Presumably, no-reflow phenomenon observed in the clinical setting, represents a situation of vascular and endothelial damage that could lead to exposure of tissue factor to the flowing blood.

From pathophysiology to therapy?

As discussed above, the exact causal chain of events leading to perfusion defects in these experimental studies has not been completely understood. As a consequence, various therapeutic approaches revealed very different, in part contradictory results. Table 1 summarizes some of the tested interventions. Even with very similar experimental approaches, results differed substantially. In addition, some interventions found to be effective in the clinical setting did not show any significant effect in animal research and vice versa. Table 2 summarizes selected interventions applied in clinical studies.

Some of the discrepancies might be explained by additional factors contributing to clinical no-reflow. As already mentioned, coronary microembolization significantly contributes to perfusion defects in acute coronary syndromes [20]. Activation of platelets and potentially the coagulation pathway might also be important, which is most likely the main underlying mechanism of beneficial effects of glycoprotein IIb/IIIa-receptor antagonists. Nonetheless, the differential contribution of various mechanisms in different situations remains to be identified to further search for effective treatment strategies. In the clinical realm, beneficial or detrimental statistical predictors of the development of no-reflow may also be identified, e. g. the baseline white cell blood count, glycemic control and control of hyperlipoproteinemia appear to significantly predict occurrence of

Table 1 Interventions used in experimental animal models to reduce no-reflow

Intervention	Species	Method for assessing no-reflow	Comment	Reference
Adenosine				
Intracoronary adenosine after reperfusion	Dogs	Radioactive microspheres, EM	Improved regional myocardial blood flow, ultrastructural preservation of endothelial cells, less neutrophils infiltration	8
Intravenous adenosine during reperfusion	Dogs	Radioactive microspheres, EM	Improved regional myocardial blood flow, ultrastructural preservation of endothelial cells, less leukocyte and erythrocyte plugging, accompanied by infarct size reduction	74
Intravenous adenosine during reperfusion	Rabbits	Thioflavin S, microspheres	No reduction of anatomical no reflow, reduction of specific vascular resistance within the risk area during reperfusion	82
Combined intracoronary adenosine and lidocaine	Dogs	Radioactive microspheres	Improved regional myocardial blood flow, accompanied by infarct size reduction, both not observed with adenosine or lidocaine alone	33
Calcium-channel antagonists				
Nisoldipine before ischemia	Rats	Fluorescein perfusion	Reduced area of no-reflow with nisoldipine in globally ischemic isolated hearts	102
Gallopamil during ischemia	Rabbits	Thioflavin S	Reduced no reflow with gallopamil during ischemia, accompanied by infarct size reduction	101
Intravenous Verapamil during reperfusion	Rabbits	Thioflavin S, microspheres	No reduction of anatomic no-reflow, reduced specific vascular resistance during reperfusion	80
Neutrophil depletion				
Reperfusion with neutrophil depleted blood (filter)	Dogs	Thioflavin S	Reduction of anatomical no reflow with concomitant infarct size reduction	62
Intraperitoneal mustin hydrochloride	Rats	Constant pressure perfusion (Langendorff)	In the abdomen transplanted hearts (global ischemia 4 hours, 4 °C), after different times of in situ reperfusion: Langendorff perfusion: better recovery of coronary flow	22
Reperfusion with intracoronary oxygenated perfluorochemical	Dogs	Thioflavin S, colored microspheres, EM, LM	Reduced area of no-reflow, less leukocyte plugging, preservation of endothelial structure	57
Oxygen-derived free radical scavenging				
Superoxide-dismutase and catalase during reperfusion	Dogs	Radioactive microspheres, EM	Preservation of endocardial regional blood flow, less ultrastructural damage to the endothelium within myocardial necrosis	75
Superoxide dismutase and catalase during reperfusion	Dogs	Radioactive microspheres	Marked hyperemic response in treated animals after 10 minutes of reperfusion	3
Active site-blocked factor VIIa				
Active site-blocked factor VIIa during reperfusion	Rabbits	Thioflavin S	Reduction of anatomical no-reflow and infarct size	27
Endothelin antagonism				
Endothelin-A antagonist during reperfusion	Dogs	Myocardial contrast echocardiography, radioactive microspheres	Enhanced microvascular flow after 180 minutes of reperfusion by contrast echocardiography and microspheres	23
Hypothermia				
Epicardial cooling during late ischemia and continued during reperfusion	Rabbits	Thioflavin S staining	Reduction of necrosis and reduction of no-reflow (with more than proportional microvascular protection)	29
Intra-aortic balloon counterpulsation				
Intra-aortic balloon counterpulsation for 24 hours after ischemia/reperfusion	Dogs	Magnetic resonance imaging	Reduced microvascular obstruction by MRI and microspheres, without reduction of infarct size	2

EM electron microscopy, *LM* light microscopy

Table 2 Interventions used in clinical situations to reduce no-reflow

Intervention	Clinical situation	Method for assessing no-reflow	Comment	Reference
Adenosine				
Intracoronary adenosine	Primary angioplasty for AMI	TIMI grade flow	Incidence of no-reflow was reduced and associated with better recovery of left ventricular function and outcome	66
Intracoronary adenosine	PTCA in AMI	TIMI grade flow	Reduced incidence of angiographic no-reflow	7
Calcium antagonists				
Intracoronary verapamil with nitroglycerine	Elective and emergency PTCA for AMI	TIMI grade flow	Improvement of TIMI grade flow after verapamil in elective and emergency PTCA	73
Intracoronary verapamil	PTCA in AMI	Myocardial contrast echocardiography	Reduction of perfusion defects and increase in peak intensity, associated with better contractile recovery	97
Intragraft verapamil and nitroglycerine	Elective PTCA in saphenous grafts	TIMI grade flow	Improvement of flow with verapamil in PTCA in saphenous grafts, in contrast: no effect of nitroglycerin	47
Papaverine				
Intracoronary papaverine	Elective PTCA in saphenous grafts	TIMI frame count	Papaverine reduced the number of TIMI frame counts in patients with no-reflow	35
Nicorandil				
Intravenous nicorandil	Anterior wall AMI	Myocardial contrast echocardiography	Lower incidence of perfusion defects in the nicorandil group	39
Nitric Oxide Donor				
Intracoronary nitroprusside	Elective PTCA	TIMI flow grade, modified TIMI frame count	Improvement of flow after nitroprusside in elective interventions	32
Glycoprotein IIb/IIIa receptor antagonism				
Intravenous abciximab	Stenting in AMI	Recovery of coronary flow velocity after 14 days	Increased improvement of coronary flow velocities and parameters of left ventricular function after myocardial infarction	70
Mechanical devices for prevention of microembolization				
PercuSurge GuardWire temporary occlusion system	Elective saphenous graft angioplaty	Prevention of microemolization	In saphenous graft interventions large amounts of artherosclerotic debris could be aspirated.	12
Dorros/Probing catheter	Elective saphenous graft angioplasty	Prevention of microemolization	The approach is feasible and debris could be aspirated	94

AMI acute myocardial infarction

no-reflow [24, 40, 56], even if a direct pathophysiologic role does not appear to be relevant. In summary, clinical observations and results of animal models should be separated closely when interpreting results and searching for mechanisms of microvascular obstruction. Nevertheless, the significant amount of no-reflow observed after coronary artery occlusion and reperfusion in animals suggests that this phenomenon also significantly contributes to clinical perfusion defects.

Myocardial necrosis and microvascular perfusion defects

In our own experiments in the rabbit, a very close correlation between myocardial infarct size and the size of anatomical no-reflow was a characteristic and reproducible feature with various experimental conditions usually resulting in correlation coefficients of more than 0.90. [78–81]. Furthermore the spatial distribution of no-reflow zones and myocardial necrosis as assessed by triphenyltetrazolium staining was very similar with zones of no-reflow tending to be slightly smaller than zones of necrosis but comprising usually more than 80 % of infarct size in this model [79, 80]. As a consequence one might conjecture that any significant infarct size reducing intervention must necessarily also result in reduction of no-reflow zones.

When testing two of the most powerful infarct size reducing interventions in these experimental models, i. e. ischemic preconditioning and inhibition of the Na^+/H^+ exchanger (by cariporide), a significant reduction in infarct size as well as no-reflow was achievable. However, when looking at the correlations between size of no-reflow and size of necrosis, it became obvious that neither of these two cardioprotective interventions,

which are believed to work independently and by separate mechanisms, differentially influenced the amount of necrosis and microvascular perfusion defects; to the contrary linear regression analysis of the size of necrosis versus size of perfusion defects did not result in significantly different interrelationships. Thus, a causal link between myocyte necrosis and microvascular perfusion defects was postulated as neither of the two interventions uncoupled necrosis from areas of no-reflow [80].

The key question that can be derived from these observations is whether the no-reflow phenomenon contributes to myocardial necrosis. While no-reflow immediately after release of the coronary artery occlusion in experimental animal models probably does not contribute to myocardial cell death [5, 27], one might argue that the marked progression of the no-reflow zone with ongoing reperfusion could be a limiting factor for the final amount of myocardial salvage. Closely linked to this question is the debate over the existence of real myocardial reperfusion injury, postulating a progression of myocyte death during reperfusion [4, 50].

One of the strongest arguments against a causal role of anatomical no-reflow for myocardial cell death during reperfusion in experimental infarction is the time-course and spatial distribution of myocyte death and vascular injury in the dog: In 312 biopsies of ischemic canine myocardium after various times of coronary occlusion (20–180 minutes), no biopsy showed microvascular damage without myocardial cell injury. In addition, ultrastructural myocyte damage markedly preceded microvascular alterations [55]. Similarly, after 5.5 hours of coronary occlusion followed by 30 minutes of reperfusion in the dog, the extent of vascular injury was always shown to be less than and well confined to the infarcted area [21].

This might be interpreted as a higher tolerance of the endothelial cells towards ischemia and reperfusion in comparison with the cardiac myocyte. One might postulate that myocardial cells, due to highly energy consuming contractile processes and complex ion-hemostatic mechanisms [46], are less resistant to ischemia and reperfusion in comparison with endothelial cells. However, contractile performance of cardiac myocytes declines very rapidly after the onset of ischemia, thus limiting energy expenditures very early in the course of insufficient oxygen supply. In addition, electron microscopic investigations after ischemia and different methods of cardioplegia rather suggested that endothelial cells might be even more prone to the ischemia/reperfusion-induced damage than cardiomyocytes [90, 91, 93].

In the early investigation on reperfusion-related expansion of no-reflow in the dog, by Ambrosio et al. "the size of infarct and low flow areas were linearly correlated . . . with a minimum infarct size of about 20 % of the risk zone required before any low flow area was seen" [5]. This could suggest that death of cardiomyocytes might be a prerequisite for no-reflow to develop. Interestingly, in this study, the finally observed areas of no-reflow were characterized by very low collateral flow during coronary occlusion.

However, in the mentioned investigations in the rabbit heart with different cardioprotective interventions, linear regression between the size of necrosis and size of perfusion defects resulted in regression lines with an ordinate-intercept near zero or even at positive values, suggesting that a certain amount of no-reflow was already present with minimal amounts of necrosis [79, 80]. These observations could also be explained by postulating that the amount of no-reflow could limit the amount of myocardial tissue to be salvaged by perfusion, thus meaning implicitly a contribution of no-reflow to myocardial necrosis. Moreover, investigations in isolated rabbit hearts using injection of low-viscosity resin were able to demonstrate zones of low-flow surrounding an area of no-reflow. Even if these low-flow areas were of small volume, they seemed to extent to non-infarcted myocardium [89], which might provide a causal link between no-reflow and myocardial necrosis.

Obviously this fundamental issue of myocardial ischemia and reperfusion cannot finally be solved on the basis of the current data. An interesting study (albeit debated with respect to its methodology [17]), using uptake of radioactive labeled derivates of deoxyglucose, administered at different time points after reperfusion, as a marker of viability, suggested that more than 50 % of biopsies taken from infarcted areas after 90 minutes of occlusion and 4 hours of reperfusion were viable after five minutes of reperfusion, but necrotic after 180 minutes of reperfusion [67]. An important finding in this study was that 97 % of the tissue samples taken from areas of no-reflow (in contrast to perfused tissue), defined as areas not perfused by thioflavin S, were judged to be already non-viable after five minutes of reperfusion, suggesting that no-reflow had not contributed to progression of myocyte death in this study.

The open-microvessel hypothesis

As there is no proof of a contribution of classical no-reflow, as observed in animal studies of coronary occlusion and reperfusion, to myocardial necrosis, one might ask, whether treatment that focuses on reduction of no-reflow makes sense. Clinical studies have documented a strong correlation between the incidence of angiographic no-reflow, perfusion defects demonstrated by contrast echocardiography, or microvascular obstruction visualized by MRI and clinical outcome [37, 107]. Interventions associated with reduction of clinical no-reflow were shown to bear prognostic benefit [39, 66]. While it is very suggestive that prevention of microembolization should be beneficial in the clinical realm [98],

it remains to be determined to what extent clinical no-reflow resembles the no-reflow phenomenon in animal research. However, the correlation between outcome and extent of clinically observed no-reflow might solely reflect the correlation of the extent of no-reflow and the size of the infarcted territory, as delineated above.

Nevertheless, improvement of tissue perfusion could have beneficial effects other than myocardial salvage: Infarct expansion, scar healing or aneurysmic ventricular dilation might be prevented by improvement of tissue perfusion even to irreversibly damaged myocytes [11]. The delivery of pharmacological agents to the myocardium would be a secondary potentially beneficial effect. In addition, blood vessels preserved in an area that might have become a no-reflow zone could serve as a source of future collateral vessels.

In a chronic model of coronary occlusion and reperfusion in the rat, it could be demonstrated that compromised tissue perfusion persists for at least four weeks. Importantly, the degree of reduced tissue perfusion correlated significantly with scar thickness and indices of infarct expansion in this study [77]. Therefore, the amount of no-reflow as present in the chronic stage, when significant steps in infarct healing have taken place, predicted infarct expansion in this study, which could be the pathoanatomic basis for the prognostic significance of no-reflow. Soon after the advent of reperfusion therapy, the open-artery hypothesis was coined, suggesting that re-opening of an infarct related artery could be beneficial with regard to infarct healing and remodeling even after significant salvage of cardiomyocytes is not achievable [49]. Looking at the interrelation between infarct expansion and no-reflow, the open-artery hypothesis might be shifted downstream to an "open-microvessel hypothesis", stressing that complete microvascular reperfusion should be the goal of true reperfusion therapy potentially resulting in beneficial attenuation of the remodeling process [77]. However, developing effective therapies will crucially depend on understanding the differential mechanisms contributing to no-reflow.

Conclusions

Both animal models of experimental myocardial infarction and clinical studies on reperfusion therapy for acute myocardial infarction, provided evidence of impaired tissue perfusion at the microvascular level after initiation of reperfusion despite adequate restoration of epicardial vessel patency. Characteristics of this "no-reflow" phenomenon found in basic science investigations, such as distinct perfusion defects, progressive decrease of resting myocardial flow with ongoing reperfusion and functional vascular alterations are paralleled by clinical observations demonstrating similar features during the course of reperfusion. In experimental animal investigations of coronary occlusion and reperfusion, this no-reflow phenomenon could be characterized as a fundamental mechanism of myocardial ischemia and reperfusion. Major determinants of the amount of no-reflow are the duration of occlusion, infarct size, but also the length of reperfusion, as rapid expansion of perfusion defects occurs during reperfusion. Moreover, no-reflow appears to persist over a period of at least four weeks, a period when major steps of infarct healing take place. The significant association of the degree of compromised tissue perfusion at four weeks and indices of infarct expansion, found in chronic animal models of reperfused myocardial infarction, might be the pathoanatomic correlate for the prognostic significance, observed in the clinical setting.

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